Drug Repurposing Option for COVID-19 with Structural Bioinformatics of Chemical Interactions Approach

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ABSTRACT

The SARS-CoV-2 virus is the pathogenic agent that caused the COVID-19 disease. The epicenter of this disease is the city of Wuhan, China. It is already categorized as "pandemic" by WHO, as many countries already affected with the infections, including recently Indonesia. Although the standard RT-PCR and DNA sequencing protocols has already developed for diagnostic, no drugs are available to cure this disease until today. The anti-malaria drug of chloroquine phosphate was repurposed, as well as other anti-viral drugs. In this regard, a structural bioinformatics pipeline was utilized to validate the claim in the computational realm. Within the sphere of the online molecular docking method, it was found that all the tested repurposed drugs attached accordingly with the SARS-CoV-2 protease enzyme that plays a role in viral replication. The repurposed drugs could be proposed as drug candidates for COVID-19, after clinical trials or further laboratory testing.

Keywords: Anti-malaria, anti-viral, COVID-19, protease, SARS-CoV2

ABSTRAK

Virus SARS-CoV-2 adalah patogen penyebab penyakit COVID-19. Episentrum penyakit ini adalah kota Wuhan, Tiongkok. WHO mengeluarkan peringatan 'pandemi' karena banyak negara sudah terkena infeksi, termasuk Indonesia. Meskipun protokol RT-PCR dan sekuensing DNA standar telah dikembangkan untuk tujuan diagnostik, hingga saat ini tidak ada obat untuk menyembuhkan penyakit ini. Obat anti-malaria *chloroquine phosphate* dicoba, bersama dengan beberapa obat anti-virus. Alur analisis bioinformatika struktural digunakan untuk validasi di ranah komputasi. Dalam lingkup metode *molecular docking* secara *online*, ditemukan bahwa obat tersebut tertambat dengan enzim protease SARS-CoV-2 yang berperan dalam replikasi virus. Obat ini dapat diusulkan sebagai kandidat obat untuk COVID-19, setelah pengujian laboratorium dan uji klinis lebih lanjut. **Arli Aditya Parikesit, Rizky Nurdiansyah. Kemungkinan *Drug Repurposing* untuk COVID-19 dengan Pendekatan *Structural Bioinformatics of Chemical Interactions***

Kata kunci: Anti-malaria, anti-viral, COVID-19, protease, SARS-CoV2

INTRODUCTION

COVID-19 disease is caused by the SARS CoV-2 virus.[1] The symptoms are respiratory symptoms, fever, cough, shortness of breath, and breathing difficulties. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and death.[2] Since the end of December 2019, the SARS CoV-2 virus had spread from the 'ground zero', the city Wuhan, China, and infected more than 50 countries.[3] Based on WHO data cohort, the current standing of COVID-19 pandemic is 179,112 infected patients, and 7,426 death (per 18th of March, 2020); mostly in China with significant occurrence in Italy, South Korea, Japan, Germany, and France. Indonesian government just recently reported 172 cases and 7 mortality in Indonesia, and the first batch of infection was reported directly by the President Joko Widodo, in the national televisions (per 10th of March 2020). Both Kemenkes (Health Ministry of Indonesia) and WHO update their COVID-19 epidemiology data cohort daily.[4,5]

Until today, there is no available drugs nor vaccine for the COVID-19. Some plausible effort to provide drug is by repurposing of the existing one, like the utilization of interferon alfa 2B by the recommendation of Cuban and Chinese scientist.[6,7] As the situation in China is improving, this approach could be leveraged to other types of drugs; based on his experience in treating previous coronavirus infection (MERS-CoV and SARS-CoV), Hongzhou Lu from Fudan University, Shanghai, and a team from Qingdao University, China, proposed to leverage existing drugs to treat the COVID-19 patients.[8] However, their claim should be validated in the clinical trial.

More encouraging news came from the field of bioinformatics, especially the structural bioinformatics field. A consortium of Chinese university has elucidated the 3D structure of the SARS CoV-2 protease. It is the first 3D structure of SARS CoV-2 protein, so it is the starting point of the investigation[9] to pave the way for drug development, and based on previous research, the drug repurposing option always provides a more feasible,

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rational, productive, and potentially lower overall development costs with shorter development timelines.[10] There is a success story of azidothymidine that failed as a chemotherapy agent, but repurposed as an anti-HIV drug.[11] Based on Hongzhou Lu and Qingdao team's literature about the drug repurposing for COVID-19, the existing drug will be tested in the bioinformatics pipeline for their fitness as lead compounds (drug candidate) for COVID-19.[12]

Table 1. QSAR annotations result from the PASS Server[26]

No.	Drug Name	Protease Inhibitor Activity	Peptidase Inhibitor Activity
1.	Chloroquine Phosphate	Yes	Yes
2.	Lopinavir	Yes	Yes
3.	Oseltamivir	NA	Yes
4.	Peramivir	Yes	Yes
5.	Remdesivir	NA	Yes
6.	Ribavirin	NA	Yes
7.	Zanamivir	Yes	Yes

Figure 1. Patchdock data of protease-oseltamivir docking analysis (PATCHDOCK).[26]

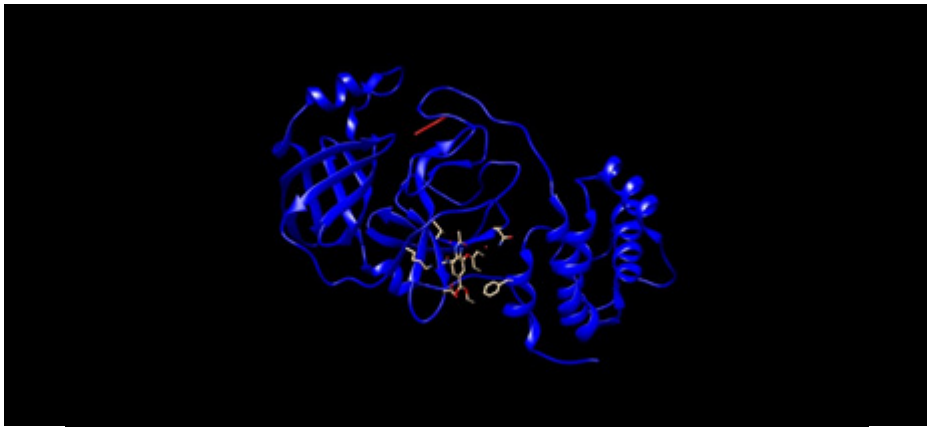

Figure 2. 3D visualization of the docking between oseltamivir and SARS CoV-2 protease (UCSF CHIMERA).[26]

The process uses the Computer-aided Drug Design (CADD) method that integrates the methods of lead compound Quantitative Structure–Activity Relationship (QSAR) optimization, sequence, and structural homology, stereochemical validation, molecular docking, and 2D molecular interaction examination.[13-15] The investigation into the sequence and structural alignment of the protease enzyme will establish the basis of the drug design.[16] The objective of this mini-research is to utilize online tools for molecular docking to screen the lead compounds' fitness as COVID-19 drug candidates from the existing drugs. These drug-repurposing efforts will eventually lead to another clinical trial session.

REVIEW OF TRIALS

The CADD method was developed by leveraging the protein sequence analysis pipeline.[17] Based on Hongzhou Lu and Quingdao's team literature, the 3D structure of the anti-viral drugs (lopinavir, oseltamivir, peramivir, remdesivir, ribavirin, and zanamivir) and anti-malaria drug (chloroquine phosphate) were downloaded from the drug bank database in PDB (Protein Data Bank) format (https://www.drugbank.ca/).[18] Thus, the drugs library was tested with the QSAR prediction with the PASS server (http://www.pharmaexpert.ru/passonline/).[19] Then, the sequence and structural alignment of the protease enzyme was searched within the automated tools in the rcsb.org.[20] The 3D structure of the first protein from SARS CoV-2 that available in RCSB (Protease Enzyme; PDB ID: 6LU7) was downloaded in PDB Format (https://www.rcsb.org/structure/6lu7). The Procheck server was employed to validate the stereochemical integrity of the 3D structure, including the generation of the Ramachandran plot.[21] The Patchdock online molecular docking program was validated with the existing example from the proteinase inhibitor structure (PDB ID: 2KAI). Then, the 3D structure of the protein was uploaded as the receptor part and the drugs as the ligand part, to the online Patchdock application (https://bioinfo3d.cs.tau.ac.il/PatchDock/index.html).[22] The final step is to load the docking result to the Ligplus application to observe the 2D molecular interaction[23,24] and then analyze the result.

DISCUSSION

The focal point of this mini-research is the molecular docking method with the online Patchdock package. Patchdock output was generated based upon the thermodynamics formula. The most spontaneous value will be the most favorable.[25] The Patchdock provides the best 10 conformations of the protein-ligand/drug, and the structural observation of the protease enzyme showed that the ligand will bind to the fittest binding site.

The very first step to predicting the feasibility of the lead compounds is determining the QSAR annotation to observe whether there will be protease and/or peptidase inhibition activity. All compounds have protease or peptidase inhibition activity prediction, and this could be an early indicator of inhibition activity in the SARS-CoV-2 protease enzyme (**Table 1**).

Herewith, the sequence alignment analysis shows that the SARS-CoV-2 protease enzyme only comprised of one chain (Chain A), and it has very high homology with SARS-CoV main protease triple mutant STI/A with two N-terminal additional residues (Gly-Ser) (PDB ID: 3M3V; http://www.rcsb.org/structure/3M3V) with these following parameters: Length: 306 E-value: 1.22336E-178 Score: 623.624bits (1607) Identities: 294/306 (96%) (Taken from: https://www.rcsb.org/pdb/explore/sequenceCluster.do?structureId=6LU7). It infers that the similarity rate with the SARS-CoV protease sequence (another type of Coronavirus) is high. Moreover, it validates the finding of the sequence analysis that it only comprises of Chain A based on structural alignment analysis. It has very high homology with Protease Do-like 9. PDB ID: 5IL9. https://www.rcsb.org/structure/5IL9 with P-Value 6.51E-5, Score 117.15, RMSD 2.49. It infers that the structure is a protease enzyme. Regarding the structural validation, the Ramachandran plot shows that there is only 1 residue in disallowed regions, that amount to 0.4 % of the total residue. It is still far below the allowed threshold of 10% which means that the protease enzyme structure is stable. The discrepancy between sequence and structural alignment occurred because the homologous regions identified by sequence comparison are often shorter than those identified by 3D structure comparison.

Table 2. The score tabulation of Patchdock docking result of the tested drug with the SARS-CoV-2 protease.[26]

No.	Tested Drug	Patchdock Scores
1.	Chloroquine Phosphate	3420
2.	Lopinavir	**6746**
3.	Oseltamivir	4412
4.	Peramivir	4214
5.	Remdesivir	**6296**
6.	Ribavirin	3464
7.	Zanamivir	4302

Table 3. Hydrogen bonding available between the ligand and the amino acid residues in the SARS-CoV-2 protease (In bold: The tested drug with the highest Patchdock scores)[26]

No.	Tested Drug	Amino Acid Residues (Sequence Number)
1.	Chloroquine Phosphate	-
2.	**Lopinavir**	Arg4, Gly2, Phe305
3.	Oseltamivir	Asp153
4.	Peramivir	Thr111
5.	**Remdesivir**	Arg188, Tyr54
6.	Ribavirin	Gly71
7.	Zanamivir	Lys10, Gln110, Asp153

The molecular docking analysis validation was conducted with Patchdock, by comparing the ACE/Gibbs free energy result of the very early iteration of the example run with the current one using the proteinase inhibitor (PDB ID: 2KAI). The score and the contact energy result are consistent with the very early iteration of the example run with less than 10% standard deviation. Thus, the molecular docking iteration was conducted with the lead compounds recommended by Hongzhou Lou and provides output as seen in **Figure 1.**

In the **Figure 1**, the important indicators that should be observed are the "NoScore", "ACE", and "PDB File of the complex". The "NoScore" and "ACE" indicators designated the free energy Gibbs iterations of the structural conformations. Hence, the "PDB file" shows the 3D visualization of the structural conformation as shown in **Figure 2**.

In **Figure 1**, the rank 1 conformation is shown as the "solution no 1" and it will be compared with each other as shown in **Table 2**. Hence, in **Figure 2**, the 3D visualization of the SARS CoV-2 Protease and oseltamivir shows that the crevice of the protein is a suitable place for the ligand docking. As seen in the 3D visualization, the protein is dominated by alpha-helix 2D confirmation, with some instances of the Beta-sheet ones (**Figure 2**).

Concerning **Table** 2, All results show negative ACE value or negative Gibbs free energy. However, lopinavir and remdesivir showed the highest Patchdock scores. It means that it has a high possibility to dock into the protease enzyme. However, more measures are needed to ensure whether the docking took place or not.

Figure 3 shows the 2D interaction of the SARS-CoV-2 protease with the default N3 inhibitor. The ligand is bound to the three amino acid residues of Thr190, Gln189, and Glu166. It states that there are hydrogen bonds occurred between the ligand and the protein. In this regard, **Table 3** shows the chemical interactions repertoire of the other lead compound.

As shown in the table, the lopinavir and remdesivir drugs have at least two hydrogen bonds with the protein.

As those drugs already have undergone clinical trials for other purposes or disease, the ADME-TOX computational prediction may not be necessary as the toxicological and pharmacological indicators already elucidated. ADME-TOX prediction should be executed if the drugs will have their functional groups modified. Moreover, it is important to note that some of the drugs already listed in the COVID-19 prevention and treatment handbook published by Zhejiang University Medical School, based upon their real clinical experience.[27]

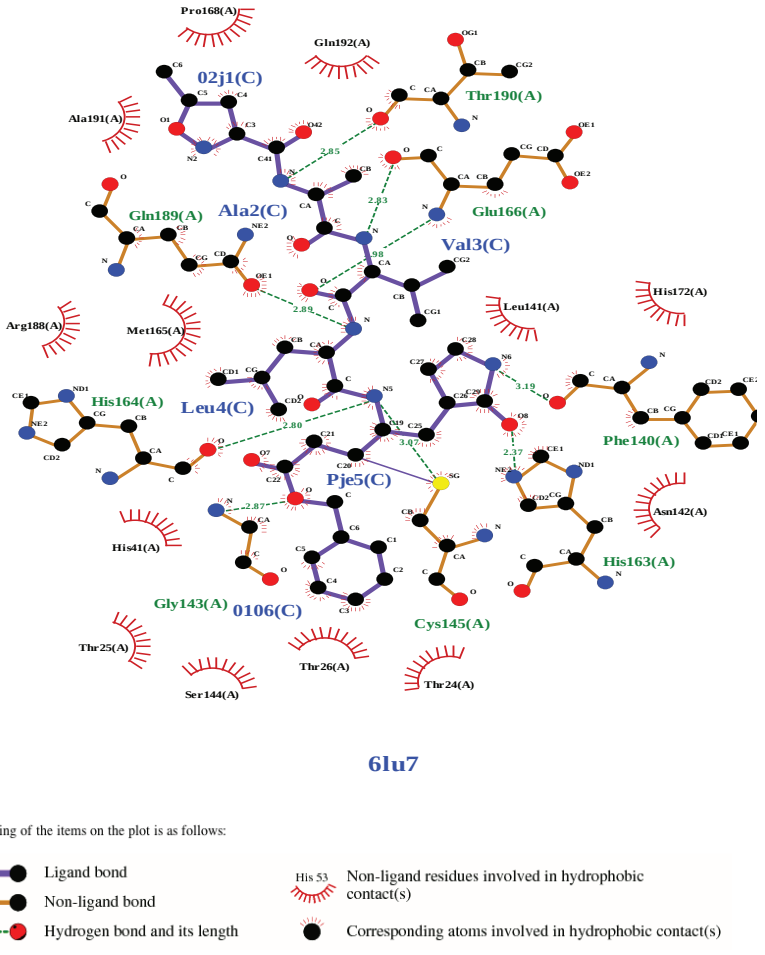

Figure 3. 2D visualization of SARS CoV-2 protease and the default N3 inhibitor (Ligplus).[26]

CONCLUSION

Based on this mini-research of structural bioinformatics, chloroquine phosphate, lopinavir, oseltamivir, peramivir, remdesivir, ribavirin, and zanamivir have the potential to be leveraged as lead compounds for COVID-19 drug candidates. Based on the docking result, the top candidates are lopinavir and remdesivir. As those drugs are repurposed, they should be revalidated in the clinical trial. In the outlook of this research bioinformatics, it is better to use offline programs such as Autodock that provide richer customization options. Moreover, the molecular dynamics method should be employed to observe the stability of protein-ligand interaction. More importantly, conducting online forum group discussion (FGD) with Chinese biomedical scientists that have experiences in drug repurposing efforts will be beneficial for information sharing. Lastly, to promote local wisdom, leveraging Indonesia-based herbal medicine could be considered for a long-term drug development strategy for COVID-19, and other coronavirus-based diseases in general.

ACKNOWLEDGMENT

The authors would like to thank the Institute for Research and Community Services of Indonesia International Institute for Life Sciences (i3l) for their heartfelt support. Thanks also go to the Indonesian Society for Bioinformatics and Biodiversity (ISBB) or *Masyarakat Bioinformatika dan Biodiversitas Indonesia* (MABBI) members and leaders for the thorough discussion on the topic of COVID-19 pandemic.

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